35 USC §102 and §103

In the absence of prior art rejections, Applicant notes the Examiner's position that the present claims are clear of the prior art.

35 USC §112 first paragraph

The terms 'fragment, analogue, variant or derivative thereof' are considered to lack enablement. The terms 'analogue' and 'derivative' are fully enabled to a person of ordinary skill in the light of the specification. However, to facilitate prosecution, they have been removed from the present application.

The terms 'fragment' and 'variant' have not been removed as they are fully enabled to a person of ordinary skill and are important in providing a scope of protection commensurate with the contribution of the present invention to the art.

A skilled person is able to make fragments of a full length polypeptide or sequence variants without undue experimental exertion. The present application teaches for the first time the binding of XRCC4 with DNA ligase IV and DNA-PKcs and describes various methods of determining this binding. In the light of this teaching, it is a trivial task for a skilled person to modify the sequence or make fragments which retain the binding activity using routine methodology, as described on page 15 lin 6 to page 16 line 23. There is no undue experimental burden in following the teaching of the application to make such minor modifications and determining whether binding occurs. For example, regions of interaction can be readily mapped using conventional techniques (see page 15 lines 6 to 19 of the present application) to enable the generation of particular polypeptide and peptide fragments which possess the binding activity of the full length protein. The present application exemplifies this process by identifying a fragment of DNA ligase IV (residues 550-844) which binds XRCC4 (page 69 line 9 to page 70 line 1).

The Examiner has raised a number of concerns regarding the significance of the experimental data in the present application, in particular in relation to the function and mechanism of action of XRCC4 and DNA ligase IV .

Whilst the function of both XRCC4 and DNA ligase IV are shown for the first time in the present application and are an important part of the teaching of the application, neither function nor mechanism are, in fact, relevant to the patentability and usefulness of the present assay method claims, in the light of the experimental demonstration in the present specification of the binding interaction.

The present findings establish that there is a strong binding interaction between DNA ligase IV and XRCC4. The specificity and strength of the observed interaction is highly indicative of the function of the complex. The interaction is very stable, since it is observed at salt concentrations well below, equal to, or far above those of the physiological state and is highly specific and selective (one protein can be used affinity-purify the other from a crude cell extract in one step).

Furthermore the two proteins ar stoichiometrically associated with one another and there are no detectable pools of each of the factors that are not associated with the other partner. That is, XRCC4 and DNA ligas IV are apparently always together with one another. This provides ov rwhelming evidence that the binding interaction is physiologically relevant.

The interaction (i.e. the binding) between the molecules has therefore been fully characterized in the specification. The demonstration of this interaction for the first time in the present application provides both support and enablement for the present claims. Compounds which modulate this binding interaction may be identified using the described assay methods without any reference to function or mechanism of action, in the same way that a pharmaceutical may be used to treat an illness without any knowledge of how the pharmacological mechanism by which the therapeutic effect is achieved.

The present specification places the XRCC4 binding interaction with DNA ligase IV into a physiological context. XRCC4 is known to be part of the Ku-associated DNA double stranded break repair (KADR) apparatus. As XRCC4 is part of the KADR apparatus and is shown to exist *in vivo* in complex with DNA ligase IV, this provides convincing evidence that both proteins are involved in KADR.

Subsequent work in the field has confirmed this conclusion. For example, disruption of the gen for DNA ligase IV in mouse leads to radiosensitivity and a defect in DNA double-strand break repair (Grawunder et al., 1998; Mol. Cell 2, 477-484; Frank et al., 1998 Nature 396, 173-177). Furthermore, mutation in DNA ligase IV in humans leads to clinical radiosensitivity and defects in DNA double-strand break repair (Riballo et al., 1999; Current Biology 9, 699- 702).

The Examiner cites the specification as indicating that the physiological role of DNA ligase IV is unknown. This is incorrect. Although the role of DNA ligase IV was unknown prior to the present work, the physiological role of DNA ligase IV is clearly established in the present specification. However, for the reasons explained above, knowledge of this role is not required for the working of the assay methods as presently claimed which are fully enabled and supported by the present specification.

Agents identified by the present methods which reduce or increase the binding of these components of the KADR apparatus are useful as modulators of the operation of the KADR pathway and therefore as potential therapeutics. These assay methods can be used to obtain suitable agents without any further elucidation of the mechanisms of KADR.

The specification is considered by the Examiner to lack guidance on the type of interaction and the extent of interaction between XRCC4 and DNA ligase IV and between XRCC4 and DNA-PKcs. This is incorrect. The binding interaction between these molecules is fully characterised in the specification (for example, page 67 line 9 to page 70 line 20 and page 91 lines 16 to 32). The skilled person is provided with all the information required to perform the claimed assay methods. In the claims, the term 'interaction' has been replaced by the term 'binding', to provide further clarification of the natur of the interaction.

Having shown that two factors bind, the modulation of that binding has a clear and definite meaning i.e. the binding is either enhanced or diminished. Ther are many known examples in which the binding of two protein factors is either enhanced or diminished by a third factor. Methods for determining such binding are well known in the art and documented in the present application.

The Examiner considers that the description is lacking in size or type of peptide fragment. Following the teaching of the present invention, a skilled person is able to generate a large number of different fragments of the full length polypeptide which are suitable for use in the present assays. A functional definition of such fragments, i.e. that they bind to the specified binding partner, is entirely reasonable to protect all these possible fragments. A third party is readily able to determine whether any peptide is a fragment of one of the described polypeptides and whether it has the required binding activity, using the methods described in the application. Fragments as defined in the present application are therefore both clear and definite without additional recitation of 'size' and 'type'.

The Examiner asserts that no substantive guidance is given regarding the claimed assay method. However, a person of ordinary skill is able to perform the assay methods as claimed using routine skill and knowledge. The invention is not limited to any particular assay format. However, various assay methods and formats are described at length in the application, for example, on page 24 lines 6 to 22 and page 29 line 1 to page 32 line 19. Contrary to the Examiner's assertion, these pages relate specifically to 'assay methods'(see for example, page 29 lines 18, 28 and 34) and provide examples of how to find a test compound and how to determine and measure binding.

Detailed technical protocols are unnecessary, given that the assay formats themselves are well-known and the experimental details (such as how much XRCC4 and DNA ligase IV to add) can be readily determined by routine methods. Indeed the experimental section described on page 68 line 24 to page 70 line 24, and page 74 line 24 to page 75 line 11, provides details of the immobilisation of XRCC4 and subsequent binding by DNA ligase IV. An assay method as described herein simply involves the presence of a test compound, an example of a test compound might be an *E. coli* lysate as described on page 70 lines 3 to 20. This is shown in the application not to modulate the binding of XRCC4 and DNA ligase IV.

The specification is therefore enabling without undue experimentation for one skilled in the art to practice the present invention commensurate with the scope of the claims.

35 USC §112 Second paragraph

Method steps have been rewritten in a numerical system to clearly identify the method steps. Lower case Roman numerals have been used to avoid confusion with the claim numbering.

Claim 1 is considered to be indefinite because it recites an 'assay method' and ends with the step of 'determining interaction'. The claim has now been amended to relate the binding to the

presence of a modulating agent.

The Examiner alleges that there is 'no indication of how the claimed compounds are measured'. The claim, of course, relates to an assay method and no compounds are claimed per se. Th present invention relates to the characterisation of novel interactions, not to a method of determining the interaction between known proteins. Many alternative ways of determining the interaction between two given proteins are well known to the person of ordinary skill (see for example page 24 lines 6-19, page 28 line 34 to page 32 line 19, page 64 line 35 to page 70 line 20 and page 91 lines 16 to 32) and may be used in accordance with the present invention. Claim 1 is a generic claim providing a generic scope of protection for the invention and it would be unreasonable to limit the claim to any particular method.

Line 1 of claim 1 has been amended to specify that the assay method is for an 'agent which modulates...' which employs a test compound. The test compound is therefore not necessarily the same as the agent.

The words 'able to' in the claims have been replaced by the term 'which modulates'.

Whilst the term 'interaction' is considered to be entirely clear in the context of protein interactions, it has been replaced by the term 'binding' in order to address the Examiner's concerns.

The acronyms XRCC4 and DNA-PKcs/Ku have been recited in full where they first appear in the claims.

The term 'including' is considered unclear because there is no indication of the amount of compound present. Claim 1 has been reworded to address this objection. A substance as describ d comprises the full-length protein or a fragment or variant thereof which binds. The essential featur is the presence of such a protein, fragment or variant in order for the assay to work. No indication of actual amounts is required as these amounts can be varied and will largely depend on technical considerations relating to the particular circumstances of a specific embodiment of the method.

A term used in a patent claim can be both generic and have a clear and definite meaning. The term 'modulate' includes both activation and inhibition, but this does not make it unclear or indefinit . The normal meaning of this term i.e. 'cause to vary' is definite and unambiguous. Further limitation to activation or inhibition would be unreasonable given that these effects, which are both provided by the present invention, are both potentially useful.

The term 'and/or', which is considered indefinite, has been replaced in the present claims by Markush wording.

The terms 'derivative', and 'analogue' have been removed from the present claims, rendering objections to these terms moot. The terms 'variant' and 'fragment' have been retained as they have a clear and definite meaning and pose no undue burden on the person of ordinary skill. The use of polypeptide fragments is described throughout the application, for example page 14 line 26 to page 17 line 1. Fragments and variants are limited in the present claims by the requirement that they possess

binding activity. The person of ordinary skill in the art is well able to make such polypeptide fragm nts and variants, given the teaching in the application of the regions of interaction, without undue experimentation.

A person of ordinary skill is able to vary an amino acid sequence and determine whether the sequence binds as described in the specification. Modification of a sequence as described on page 16 lines 1 to 23 to obtain a variant with binding activity does not present an undue burden of experimentation, nor is it indefinite or unclear. A skilled person is readily able to determine whether a particular polypeptide is a sequence variant as described which has the stated binding activity.

Claim 3 has been amended to specify that the assay method is for 'an agent which modulates' This renders moot any concerns the Examiner has with the term 'affect'. 'Modulate' is a term with a clear and definite meaning, as described above.

The Examiner's rejection of Claim 4 is not understood. The claim recites a method "wherein the DNA ligase activity is determined in the presence of XRCC4", and does not refer to a SEQ ID NO. Clarification is requested.

Claim 5 has been re-worded to recite positive method steps. The term 'able to' has been removed to address the Examiner's concerns.

The term 'join' is not indefinite and is used in the art to refer specifically to the end to nd connection of DNA strands, for example by a ligation reaction. The term does not include the annealing of complementary strands. To further clarify this point, the term 'end-joining' has been used in the amended claims. Basis for this wording is found on page 7 line 13 of the specification.

Claim 6 has been re-worded to removed the term 'suitable peptide', rendering moot objections to this term. The term 'phosphorylates' is now used correctly.

Claims 19, 22 and 25 have been re-worded to remove the allegedly indefinite wording.

Conclusion

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of tim , or credit any overpayment to Deposit Account No. 50-0815, order number MEWE005.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: May 8, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend claims 1 to 6, 19 , 22 and 25 as follows;

1. An assay method for [a compound able to modulate] an agent which modulates the [interaction or] binding between XRCC4 (XR-1 Cell Complementing 4) and DNA ligase IV, or XRCC4 and DNA-PK_{cs}/Ku (DNA-dependent Protein Kinase catalytic subunit/Ku) , or XRCC4, DNA ligase IV and DNA-PK_{cs}/Ku, the method comprising [including] the steps of:

[(a)](i) bringing into contact a substance which comprises [including] XRCC4 or a peptide fragment of XRCC4 or a [derivative,] variant [or analogue] thereof [able to bind] which binds DNA ligase IV or DNA-PK_{cs}/Ku, a substance [including] which comprises one or more components selected from the group consisting of DNA ligase IV or a peptide fragment of DNA ligase IV or a variant[, derivative or analogue] thereof [able to bind] which binds XRCC4 and [and/or including] DNA-PK_{cs}/Ku or a peptide fragment of DNA-PK_{cs}/Ku or a variant[, derivative or analogue] thereof [able to bind] which binds XRCC4;

and a test compound under conditions wherein, in the absence of said test compound being an inhibitor of [interaction or] binding between said substances, said substances [interact or] bind; and

[(b)] (ii) determining [interaction or] binding between said substances,

reduction or abolition in binding between said substances being indicative of said test compound being an agent which modulates binding between XRCC4 and DNA ligase IV, or XRCC4 and DNA-PK_{cs}/Ku or XRCC4, DNA ligase IV and DNA-PK_{cs}/Ku.

2. An assay method for [a compound able to modulate] an agent which modulates [interaction or] binding between XRCC4 and DNA ligase IV or XRCC4 and DNA-PK_{cs}/Ku, or XRCC4, DNA ligase IV and DNA-PK_{cs}/Ku, the method [including] comprising the steps of:

[(a)] (i) bringing into contact a substance [including] which comprises XRCC4 or a peptide fragment of XRCC4 which [interacts with] binds DNA ligase IV or DNA-PK_{cs}/Ku, or a [derivative,] variant [or analogue] thereof which binds [interacts with] DNA ligase IV or DNA-PK_{cs}/Ku, or [which includes] which comprises DNA ligase IV or DNA-PK_{cs}/Ku or a peptide fragment of DNA ligase IV or DNA-PK_{cs}/Ku which [interacts with] binds XRCC4, or a [derivative], variant [or analogue] thereof which [interacts with] binds XRCC4, and a test compound; and,

[(b)] (ii) determining [interaction or] binding between said [substances] substance and [the] said

test compound,

binding between said substance and said test compound being indicative of said test compound being an agent which modulates binding between XRCC4 and DNA ligase IV or XRCC4 and DNA-PK_{cs}/Ku or XRCC4, DNA ligase IV and DNA-PK_{cs}/Ku.

3. An assay method for [a compound able to affect] an agent which modulates DNA ligas IV activity, the method including the steps of:

[(a)] (i) bringing into contact DNA ligase IV and a test compound; and

[(b)] (ii) determining DNA ligase activity in the presence and absence of test compound,

a difference in activity in the presence relative to the absence of test compound being indicative of said test compound being an agent which modulates the activity of DNA ligase IV

5. An assay method according to Claim 4 wherein the activity of DNA ligase is determin d by determining [its] adenylation or labelling of said ligase using ATP or an ATP analogue or [ability to join] by determining end-joining of strands of DNA or DNA analogues.

6. An assay method including

[(a)] (i) bringing into contact a substance which includes DNA-PKcs/Ku or a [suitable] peptide fragment of DNA-PKcs/Ku or [derivative,] variant [or analogue] thereof [able to] which phosphorylates XRCC4, a substance which includes XRCC4 or a peptide fragment of XRCC4 or a [derivative,] variant [or analogue] thereof including a site phosphorylated by DNA-PKcs, and a test compound; and

[(b)](ii) determining phosphorylation at said site in the presence and absence of test compound

a difference in phosphorylation in the presence relative to the absence of test compound being indicative of said test compound being an agent which modulates the phosphorylation of XRCC4 by DNA-PK_{cs}/Ku.

19. A method [which includes, following] comprising obtaining [a compound] an agent able to modulate the [interaction or] binding between XRCC4 and DNA ligase IV, or XRCC4 and DNA-PK_{cs}/Ku, or XRCC4 and DNA ligase IV and DNA-PK_{cs}/Ku, employing a method according to claim 1 or claim 2; and, formulating [the compound] said agent into a composition including a pharmaceutically acceptable excipient.

22. A method [which includes, following] comprising obtaining [a compound able to affect] an agent which modulates DNA ligase IV activity employing a method according to Claim 3 and formulating said agent [the compound] into a composition including a pharmaceutically acceptable

excipient.

25. A method [which includes, following] comprising obtaining [a compound] an ag nt which modulates [able to affect] DNA-PKcs/Ku phosphorylation of XRCC4 employing a method according to claim 6 and formulating [the compound] said agent into a composition including a pharmaceutically acceptable excipient.